Grouping reduces the metabolic demand of a social squid

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ABSTRACT: Many squids are social cephalopods and demonstrate exceptionally high metabolic rates. However, all prior investigations of metabolism in social cephalopods have utilized individual animals. We measured oxygen consumption of the social squid *Doryteuthis opalescens* both in groups and with solitary individuals to examine the influence of grouping on energy demand and performance under hypoxic conditions. On average, the presence of conspecifics reduced routine metabolic rate by 21% but did not influence the critical oxygen partial pressure below which a stable rate of oxygen uptake is not maintained. In addition, displays of chromatic behaviors associated with relaxation were observed more frequently in groups, whereas behaviors associated with stress or vigilance were observed more frequently in solitary individuals. We hypothesize that through a potential reduction in energy consumption, grouping may be relevant in allowing social squids to exploit habitats with marginal oxygen availability that may impose a physiological constraint.

KEY WORDS: Social facilitation · Metabolism · Group dynamics · Oxygen consumption · Hypoxia · Cephalopod · Squid

1. INTRODUCTION

Grouping is a common feature of numerous animal taxa that can confer diverse benefits, including protection (Seghers 1974, Pulliam & Caraco 1984, Molvar & Bowyer 1994). In some cases, the presence of conspecifics can also decrease stress and energy consumption, presumably because it minimizes the need for individuals to be vigilant (Martin et al. 1980, Hennessy et al. 2009). For instance, the routine metabolic demand of several species of social marine fishes, i.e. species that form interacting shoals that exhibit antipredator and foraging group behaviors (Pitcher 1983), is substantially reduced (27–57%) when individuals are in the presence of conspecifics compared to individuals in isolation (Parker 1973, Nadler et al. 2016). However, the extent to which grouping influences energy demand has not been investigated in other social marine taxa.

Squid are abundant, highly motile cephalopods that arguably constitute one of the largest predatory biomasses on the planet (Boyle & Rodhouse 2005). Exceptionally high metabolic rates, in addition to other physiological constraints including energetically costly locomotion and limited blood oxygen carrying capacity (O'Dor & Webber 1986, Pörtner 2002, Seibel 2016), suggest that some squids may not be adaptively suited to oceanographic shifts in oxygen availability (Rosa & Seibel 2008, Seibel 2015, Breitberg et al. 2018). To our knowledge, all previous measurements of metabolic rate in squid have involved experiments on individual animals, even though many of the squids studied spend the vast majority of their lives in groups (Table S1 in Supplement 1 at www.int-res.com/articles/suppl/m612p141_supp/). There are often substantial differences in metabolic rates between seemingly similar individu-

als of a given species (McLean et al. 2018), and grouping is thought to dampen this individual variability (Burton et al. 2011). As in the case of fish (Parker 1973, Nadler et al. 2016), incorporating grouping behavior into laboratory studies may provide a more ecologically relevant estimate of squid metabolic demand, particularly in regard to environmentally driven changes in spatial distributions that may expose the animals to hypoxic environments (Deutsch et al. 2015).

We used swim tunnel respirometry to investigate how grouping is related to metabolism in the California market squid *Doryteuthis opalescens*, a nearshore social loliginid (Fig. 1) (Zeidberg 2013). Like many other squids, *D. opalescens* is thought to regularly operate near functional limits in terms of environmental oxygen availability (O'Dor & Webber 1986, Pörtner 2002, Seibel 2016): it engages in high-intensity aerobic behaviors under normoxic conditions (O'Dor 1988, Zeidberg 2004) and encounters regions of acute hypoxia during vertical migrations (Stewart et al. 2014). We compared energy demand under routine activity (routine metabolic rate, RMR) measured in solitary individuals versus groups. As a measure of physiological performance under hypoxia, we also measured the critical oxygen partial pressure below which a stable rate of oxygen uptake could not be maintained (P_{crit}) (Rogers et al. 2016) for comparison between solitary individuals and groups. Based on studies of fish (Parker 1973, Nadler et al. 2016), we hypothesized that RMR would be reduced when squid were in the presence of conspecifics.

Fig. 1. Group of California market squid *Doryteuthis opalescens* in captivity. Photo credit: Diana Li (used with permission)

2. MATERIALS AND METHODS

2.1. Specimen capture

Doryteuthis opalescens was captured over spawning grounds in nearshore waters of Monterey Bay, CA, from June to November 2017 using luminescent barbless jigs. Only undamaged squid caught by the sucker cups on the arms were retained for this study. After capture, specimens were immediately transported in aerated seawater to an indoor aquarium facility at Hopkins Marine Station, Pacific Grove, CA. There they were kept together, typically in a group of 20 to 40 animals, for 24 to 72 h in a 3200 l circular tank with flow-through seawater (20 l min^{-1}) at ambient temperature (13–16°C) and fed live rosy red fathead minnows *Pimephales promelas* once daily. Although these fish are not natural prey for *D. opalescens*, they provide sufficient sustenance for short periods in captivity (Fiorito et al. 2015). Prior to each experiment, squid were not fed for 24 h. No injuries were observed in any animals used in experiments. Standard morphometric measurements, including dorsal mantle length, mantle diameter at the widest point, wet weight, and sex, were recorded for all experimental specimens (Table S2 in Supplement 2). Squid mass in our experiments ranged from 17.4 to 81.1 g, with an average (±SD) of 44.1 ± 13.6 g; both males (n = 150) and females (n = 23) were used. All squid used in this study were mature.

2.2. Respirometry experiments

Squid from holding tanks were arbitrarily (i.e. with no particular criteria) assigned to solitary or group treatments. Fourteen solitary (1 squid) and 14 group (8–13 squid) experiments were conducted in 10 and 185 l swim tunnel respirometers (Loligo Systems), respectively. Each respirometer was immersed in a large buffer tank filled with flow-through ambient seawater for temperature control. Test sections for respirometry measurements had dimensions of 40 × 10 × 10 cm (4 l volume) in the small and 87.5 × 25 × 25 cm (54.7 l volume) in the large respirometer. Squid were individually introduced to the test section tail first

and allowed to adjust for 1.5 h (the average duration used in previous studies of squid, Table S1 in Supplement 1) with aerated seawater from the buffer tank flowing through at a rate of 3 to 5 l min^{-1}. To ensure mixing during closed respirometry, water was circulated in both respirometers at a rate of 5.3 to 6 cm s^{-1} after correcting for the blocking of flow by test animals (Brett 1964, Bell & Terhune 1970). These flows were not sufficient to force squid to actively swim to maintain position, and they were able to hover, swim forward or backward, or rest on the bottom.

All closed respirometry experiments occurred between sunrise and sunset under the same lighting condition with an average duration of 5.5 h. Respirometers were visually shielded with opaque plastic sheeting suspended over the tank and enclosing the circumference, but squid could be observed through a small opening without disturbance to ascertain skin color changes generated by chromatophores as discussed in Section 2.6. When the oxygen concentration reached 0.5 mg l^{-1}, or squid started showing obvious signs of fatigue, the experiment was terminated, and animals were removed and euthanized following sedation in a 1 to 2% solution of ethanol in seawater to ascertain morphometric measurements.

Oxygen concentration and saturation were recorded in the large respirometer at 0.1 Hz with a HQ40D multimeter and LDO101 oxygen sensor (Hach) and at 1 Hz in the small respirometer with a Witrox 1 oxygen meter and dipping probe mini sensor (Loligo Systems). For consistency, both instruments were simultaneously calibrated immediately before each experiment under the same atmospheric pressure, temperature, and salinity conditions to 0% oxygen saturation using 1 to 2% sodium sulfite in seawater and to 100% using seawater mixed for 10 min on a magnetic stir plate.

Experiments were conducted at the temperature of the ambient flow-through seawater that ranged from 13 to 16°C over the course of the study, but during an experiment, temperature in the respirometers rarely fluctuated by more than 0.1°C. Average experimental temperature (14.6 and 15.0°C in group and solitary, respectively) was not different between treatments (p = 0.2, 2-sample t-test). The Hach multimeter was capable of live temperature and atmospheric pressure correction, and we manually entered these corrections into the Witrox meter, using atmospheric pressure recorded by the Hach multimeter and temperature measured with a digital thermometer (51 II, Fluke). None of these small variations within single experiments substantially altered oxygen values or calculated uptake rates.

Control experiments containing no squid were conducted to quantify microbial background respiration. In the 185 l swim tunnel, no background respiration over experimentally relevant durations was detected, and in the 10 l tunnel, the background rate was negligible (less than 0.1% of squid rates). Therefore, we did not perform any correction to specimen rates for background respiration.

Although we did not measure CO_2 concentration (or pH) during experiments, the buildup of this metabolic byproduct is unavoidable in closed-system respirometry. The high pH sensitivity of hemocyanin, the respiratory protein of squid, has led to the concern that changes in seawater pH may impact squid metabolism (Rosa & Seibel 2008, Seibel 2016). However, recent studies examining the loliginid squids *Sepioteuthis lessoniana* and *D. pealeii* have reported no effect of lowered pH on metabolic rate and P_{crit}, presumably due to the regulation of blood pH (Hu et al. 2014, Birk et al. 2018). Wood (2018) suggests that closed-system respirometry is preferred because it is more ecologically realistic, as natural hypoxia typically involves simultaneous CO_2 buildup.

2.3. Data analysis

We analyzed data in R (R Core Team 2018), primarily using functions in the package respR (Harianto et al. 2019). Unless otherwise noted, all tests described herein were 2-tailed and met appropriate test assumptions.

2.4. RMR

RMR for both solitary squid and groups was calculated as the lowest rate sustained during 40 min over a specific range of oxygen concentration (7–5 mg O_2 l^{-1}) that was well above P_{crit} and where the oxygen uptake rate was relatively consistent (Fig. 2). The average time after an experiment started when RMR was determined (1.6 and 1.7 h in groups and solitary animals, respectively) was not different between groups or solitary squid (p = 0.65, 2-sample t-test). RMR was therefore determined over 3 h after any handling had occurred. Respiration rates for most specimens were consistent from experiment start until P_{crit} was reached and so remained stable for 4.5 to 7 h after handling. As in other studies of the metabolism of squid (Table S1) and fish (Parker 1973, Herskin 1999, Nadler et al. 2016), we used wet weight to determine a mass-specific RMR.
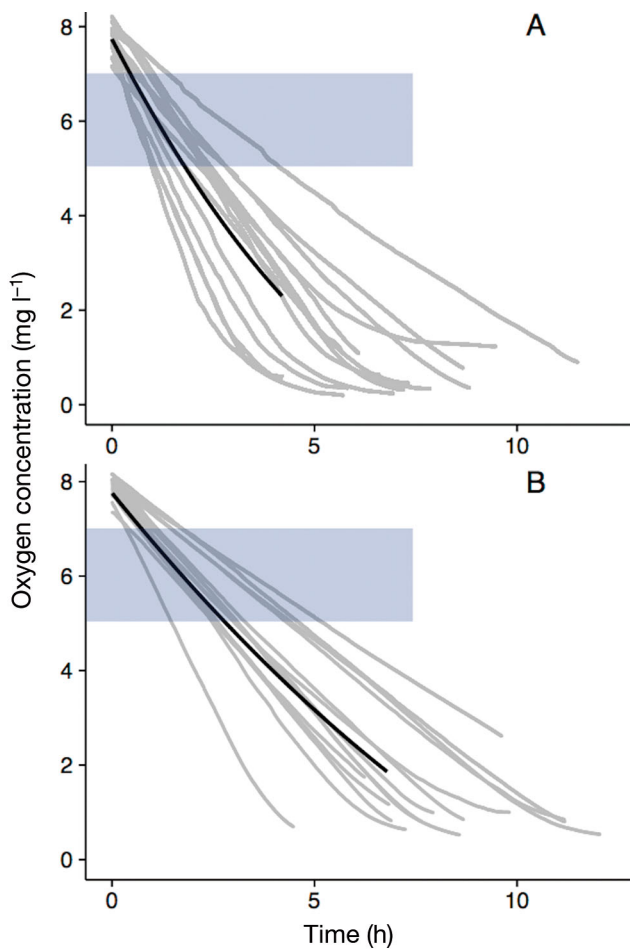
Fig. 2. Oxygen concentration in respirometers over time during experiments with (A) solitary squid and (B) grouped squid. Grey lines represent the oxygen traces of each experiment, and black lines are the smoothed averages of all data points from experiment start to the average time at which P_{crit}, the critical oxygen partial pressure, was reached in each social condition treatment (4.2 h in solitary and 6.8 h in grouped squid). The blue rectangle highlights the range of oxygen concentration (7–5 mg l^{-1}) over which we calculated routine metabolic rate (RMR) as the lowest oxygen consumption rate over a 40 min period

For solitary individuals, we normalized the measured rate to a standard mass (44.1 g) using the mass-scaling exponent for the family Loliginidae (b = 0.916) (Seibel 2007). For each group, we estimated the normalization constant, or intercept (b_0) of the rate–mass power relationship (Eq. 1),

$$b_0 = \frac{B}{\sum_{i=1}^{n} M_i^b} \qquad (1)$$

by apportioning total group RMR (B) among individuals within the group based on their masses (M) using the same mass-scaling exponent (b). The

resulting mass–metabolic rate equations (in the format of Eq. 1, Seibel 2007) then calculated the effective rate for a single idealized squid from each group at the standard weight of 44.1 g. Mass-normalized RMR was therefore compared between 14 squid in groups and 14 solitary squid (Table 3 in Supplement 2).

The first goal of this study was to determine if squid in groups consumed less oxygen per unit mass than solitary squid, the null hypothesis being that squid in groups did not consume oxygen at a lower mass-specific rate. The directional nature of this question, which we based on what is known from studies of fish (Parker 1973, Nadler et al. 2016), warranted 1-tailed testing. We therefore used an ANCOVA, a test that relies on the F distribution and is therefore 1 tailed, to compare mass-normalized RMR between social condition treatments while removing variation attributable to differences in experimental temperature (Fig. 3A, Table A1 in the Appendix).

Variance in the RMR values for grouped squid was lower than that for solitary squid, a phenomenon also noted in studies of fish (Burton et al. 2011). This suggests that squid were more relaxed within groups, maintaining more consistent RMRs and therefore displaying lower variance in group RMR. However, it is also possible this is a result of a dampening effect where individuals within groups displayed similarly high and low RMRs, but this variance was masked as a result of examining them as a group. To further assess if RMR was lower in groups compared with solitary squid, while accounting for the possibility that grouped squid had the same RMR variance as solitary squid, we adjusted the mass-normalized RMR of solitary and grouped squid to a standard temperature (14°C) using the squid-specific temperature coefficient (Q10) of 2.0 (Rosa & Seibel 2010, Trueblood & Seibel 2013). We then compared averages using a 1-tailed 2-sample t-test, with the variance for grouped squid set equal to the larger value for solitary squid.

2.5. P_{crit}

The second goal of this study was to determine if there was a difference in hypoxia tolerance between squid in groups and solitary squid. To accomplish this, we used a segmented regression approach to calculate the breakpoint of oxygen consumption curves (Muggeo 2003) to determine P_{crit}, the oxygen partial pressure below which a stable rate of oxygen uptake is not maintained (Rogers et al. 2016). In 2 individuals, P_{crit} could not be reliably determined
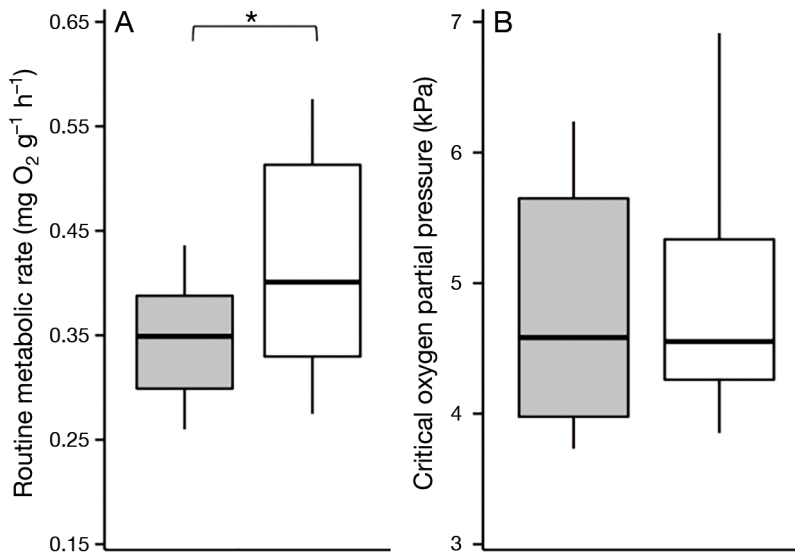
Fig. 3. Influence of grouping on metabolic features of the California market squid *Doryteuthis opalescens*. (A) Mass-normalized routine metabolic rate (RMR) (mean ± SD) estimated for single squid within groups (gray) was 0.34 ± 0.057 mg O_2 g^{-1} h^{-1} versus 0.43 ± 0.12 mg O_2 g^{-1} h^{-1} measured for individuals isolated from conspecifics (white). (B) Critical oxygen partial pressure (P_{crit}) (mean ± SD) was 4.96 ± 1.03 kPa in squid groups (gray) and 4.79 ± 0.98 kPa in solitary animals (white). In both, dark horizontal lines show the median value, with boxes and vertical lines respectively representing the 2 inner and outer quartiles of the data. *Significant difference between means (α = 0.05)

because of the variable nature of the data, and these individuals were excluded from the analysis. In 2 groups, experiments were not of sufficient duration to reach a definitive P_{crit}, and these were also excluded. This left 12 P_{crit} estimates in each social condition treatment that were examined using a 2-sample *t*-test (Fig. 3B). Because of differences in relative volume of the respirometers, groups tended to take an average time of 6.8 h to reach P_{crit} compared to 4.2 h in solitary squid.

2.6. Chromatic behaviors

To provide a behavioral context for the comparison of metabolic features, we assessed differences in the chromatic body patterning of squid in groups versus solitary squid during experiments. During the respirometry trials, we observed the behavior of solitary squid and an arbitrarily selected individual within all groups once every 1 to 3 h for 1 min and recorded all chromatic displays described for this species (Hurley 1977, Hunt et al. 2000) as being present or absent during each observation period (Hunt et al. 2000, Bush et al. 2009, Burford et al. 2015). The frequencies of the 3 most commonly performed chro-

matic behaviors, countershading, pale (clear), and dark arms (Hurley 1977, Hunt et al. 2000), were calculated for each observed squid by dividing the number of occasions each pattern was displayed by the total number of observations in that experiment. If fewer than 3 total behavioral observations were made for a given solitary squid or group, the corresponding behavioral data were excluded from analysis. This left 97 observations of 13 groups and 56 observations of 11 solitary squid, over 2.5 h of cumulative behavioral observation. For comparison, the behavioral ethogram established for *D. opalescens* was based on 2 h of behavioral footage (Hunt et al. 2000). Although we did not directly quantify it, the duration that any pattern is displayed is also an important feature of squid chromatic behavior (Hanlon & Messenger 2018).

To examine how social condition was related to the frequencies of each behavior, which were non-independent measures, we performed 3 linear mixed effects analyses with the frequency of the different displays (countershading, pale, and dark arms) as the dependent variable and social condition (group or solitary) as the independent variable. Social condition was the fixed effect, and to account for variation among groups and individuals, by-group and by-individual intercepts for the influence of social condition on behavioral frequency were random effects. We plotted the frequency of each chromatic pattern versus social condition (0 = group and 1 = solitary) and used the slope as determined by linear mixed effects analyses to assess the extent to which each behavior was associated with social condition (Fig. 4).

3. RESULTS AND DISCUSSION

3.1. RMR and chromatic behaviors

Previous studies of squid metabolism have all utilized individual animals (Table S1 in Supplement 1), and to ascertain the effect of grouping on metabolic demand in *Doryteuthis opalescens*, a social squid, we studied both solitary individuals and grouped animals. We found that the mass-normalized RMR of grouped squid (0.34 ± 0.057 mg O_2 g^{-1} h^{-1}, mean ±
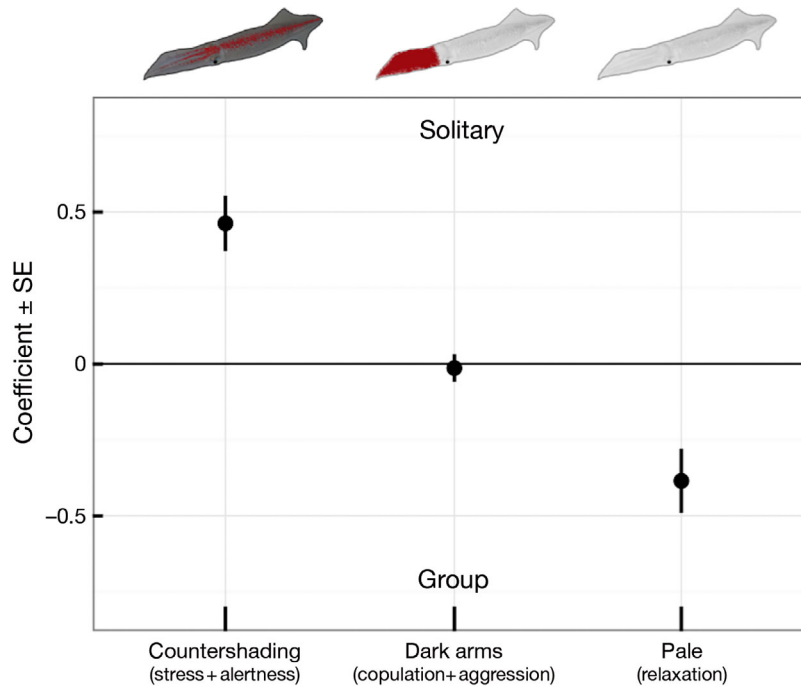
Fig. 4. Association between social condition and the 3 most commonly exhibited chromatic behaviors of *Doryteuthis opalescens* observed during respirometry experiments. Coefficients represent the slope of linear mixed effects analyses relating the frequency of each behavior to social condition while taking into account potential differences in the baseline behavioral tendencies of each individual or group observed during experiments. Solitary squid displayed countershading more frequently than squid within groups (p < 0.005), which displayed pale coloring more frequently than solitary squid (p = 0.0014); the frequency of dark arms was not related to social condition (p = 0.77)

SD) was lower than that of solitary squid (0.43 ± 0.12 mg O_2 g^{-1} h^{-1}) by 20.9% (0.09 mg O_2 g^{-1} h^{-1}, p = 0.013, ANCOVA) (Fig. 3A, Table A1). This difference was also significant when the comparison was made of grouped versus individual squid with the variance in both cases set to the larger value from the individual experiments (p = 0.028, 1-tailed 2-sample *t*-test). Sex composition had no apparent influence on group RMR (Fig. A1, Table A1), and neither did group size (range 8–13) (−0.001 mg O_2 g^{-1} h^{-2} squid^{-1}, p = 0.89, linear regression).

Squid in groups also exhibited pale coloration more frequently during the behavioral observations, whereas those that were isolated from conspecifics more often exhibited darker coloration (Fig. 4). Pale coloration in all coleoid cephalopods is due to a relaxed state of the radial muscle fibers that control the size of chromatophores, and in *D. opalescens* and many other species, pale skin color is associated with a relaxed condition of the whole animal, in contrast to dark coloration that indicates alertness or vigilance (Hunt et al. 2000, Hanlon et al. 1994, Hanlon & Mes-

senger 2018). Thus, grouping in *D. opalescens*, a naturally social species, probably reduces stress, and this factor could influence metabolic demand.

Mechanisms of energy saving by grouping (Krause 1994) include hydrodynamic effects in schooling fish (Marras et al. 2015). In our experiments, a significant contribution from this effect is unlikely, because water was circulated in the respirometers at very low flow rates (5.3–6 cm s^{-1}). This notion is supported by Payne et al. (2011), who found that metabolic rate and body acceleration of giant Australian cuttlefish *Sepia apama* under similar flow rates were not different than under no flow. Because cuttlefish and squid have different swimming kinematics (the former mainly uses finning; the latter uses a combination of finning and jetting), direct comparison between these taxa is problematic. However, we saw little indication during our experiments that movements of *D. opalescens* at these low flow rates were any different to those observed in more static water in the larger holding tanks (B. P. Burford & N. Carey pers. obs.). During our experiments, squid did not swim in a unidirectional manner with or against the current, and movements were always slow in comparison to peak velocities that this species can attain (140 cm s^{-1} or ~10 mantle lengths s^{-1}) (O'Dor 1988, Neumeister et al. 2000). Nonetheless, the level of activity was higher than that associated with standard or basal metabolic rate, and the variance of our measured rates may reflect slight differences in activity level. If hydrodynamic effects are relevant to the energetics of squid grouping, it is likely that this is true only at much faster swimming speeds than those used in our study.

Not all group-forming animals experience a reduction in energy demand from social stimuli (Herskin 1999). Within those that do, there are a multitude of factors that can alter the degree to which conspecifics calm group members. For instance, some social species acclimate to novel environments, and thus anxiety can be reduced as an experiment progresses (Hennessy et al. 2009). To ensure that our results were comparable with previous work, the time allowed for adjustment to experimental conditions was kept similar to that in other metabolic studies of

social cephalopods (Table S1). Following the period of adjustment to the respirometers (1.5 h), the average time elapsed during experiments before RMR was measured had a strong negative association with group RMR (-0.08 mg O_2 g^{-1} h^{-2}, p = 0.00059, linear regression) and no relationship with solitary squid RMR. This suggests that groups adjust to experimental conditions faster than do solitary squid, as lone squid must maintain heightened vigilance.

Under natural conditions, many active squids including *D. opalescens* are thought to rapidly transition between different regions of the water column that markedly differ in irradiance levels, hydrographic properties, and the relative abundance and composition of predators, prey, and competitors (Santora et al. 2012, Zeidberg 2013). We hypothesize that interactions with conspecifics could facilitate the exploration and occupation of diverse habitats, potentially by reducing stress-associated metabolic reactions to novel stimuli.

3.2. P_{crit} and hypoxia tolerance

We found P_{crit} (mean ± SD) in solitary and grouped *D. opalescens* to be 4.96 ± 1.03 and 4.79 ± 0.98 kPa, respectively, and hypoxia tolerance as measured (P_{crit}) was not significantly different between social condition treatments (p = 0.67, 2-sample *t*-test) (Fig. 3B). In some fish, hypoxia tolerance can be increased (as indicated by a reduction in P_{crit}) through acclimatory responses to environmental hypoxia (Timmerman & Chapman 2004) such as reversible gill remodeling (Gilmour & Perry 2018). Temperature can also modify hypoxia tolerance in some fish by altering physiological processes (Pörtner & Lannig 2009), and for most species that have been studied, P_{crit} occurs at higher oxygen concentrations at higher temperatures (Rogers et al. 2016). At present, we have no information on this subject in regard to squid.

D. opalescens migrates both vertically and horizontally in the California Current (Zeidberg 2013), an ecosystem that is spatially and temporally heterogeneous in terms of temperature and oxygen concentration (Checkley & Barth 2009). Its nearshore spawning areas are also subject to highly dynamic changes in hydrographic properties, including upwelling-related shifts in temperature and oxygen concentration (Booth et al. 2012). We would thus expect our sampling period (June–November) to be associated with some degree of seasonal, weekly, and daily variation of water column properties.

Moreover, squid collected on the spawning grounds probably migrated to Monterey Bay from a variety of offshore locations in the California Current. In either case, animals might have acclimated to different hydrographic conditions before capture. We found that measured P_{crit} values did not depend on the month of capture (p = 0.81, ANOVA), suggesting that the integrated environmental history of the squid we sampled was reasonably consistent or that acclimation may not be relevant to hypoxic tolerance in *D. opalescens* as manifested in P_{crit}.

Oxygen concentration on the spawning grounds for *D. opalescens* in Monterey Bay rarely falls to a level below that corresponding to P_{crit} (1.9–2.0 mg l^{-1}) (Booth et al. 2012). We might therefore expect that spawning adults are probably not seriously threatened by such hypoxic events, at least as defined by the P_{crit} criterion. On the other hand, juveniles of this species are regularly exposed to lower temperatures (5–8°C) and oxygen concentrations (0.7–2.1 mg l^{-1}) in offshore waters during excursions to depths of ~300 m (Zeidberg 2013, Stewart et al. 2014). Our observed value for the concentration equivalent of P_{crit} is thus higher than the oxygen concentration at this lower depth, but how P_{crit} might be affected by the lower temperature is not known for this species.

4. CONCLUSIONS

Many group-forming cephalopods, including *Doryteuthis opalescens*, are ecologically and economically important (Arkhipkin et al. 2015). Like other pelagic taxa, they are subject to oceanic changes that impact metabolism, specifically warming and deoxygenation (Levitus et al. 2000, Stramma et al. 2008). Generally high metabolic rates, energetically costly jetting, and low blood oxygen carrying capacity in pelagic squids have been suggested to make this group unduly sensitive to these ongoing changes (O'Dor & Webber 1986, Pörtner 2002, Rosa & Seibel 2008, Seibel 2016), but physiological and behavioral adaptations may serve as buffers to such challenges. For example, the Humboldt squid *Dosidicus gigas* greatly reduces its RMR in the face of severe hypoxia (Gilly et al. 2006, Rosa & Seibel 2010) and engages in long bouts of climb-and-glide swimming (Gilly et al. 2012), both of which allow it to spend the majority of daytime hours at severely hypoxic depths associated with the oxygen minimum zone (Stewart et al. 2014). Our results are consistent with the idea that grouping squid may have a lower RMR, because they are less stressed than isolated individuals (Parker 1973, Mar-

tin et al. 1980, Hennessy et al. 2009, Nadler et al. 2016). Given the lower overall metabolic rates in groups, constraints related to oxygen supply may be less likely to restrict the use of hypoxic zones.

In theory, an average energetic savings of 21% across a squid's lifetime conferred by the calming effect of conspecifics could directly relate to enhanced foraging and predator evasion (Williams et al. 2000), because squid would have more resources available to divert to these tasks. This could potentially enhance competitive ability under changing ocean conditions (Breitberg et al. 2018). However, our results additionally suggest that individual squid separated from social groups likely incur substantial metabolic consequences. A reliance on conspecifics for protection may therefore be tied to the maintenance of group cohesion during directed collective movements (Hanlon & Messenger 2018). It remains unknown how changes in environmental oxygen availability impact the spatial organization capabilities of squid groups.

Deutsch et al. (2015) defined a metabolic index model to serve as a tool for predicting shifts in marine ectotherm distribution resulting from changes in oxygen availability. This model relies on the lab-derived relationship between hypoxia tolerance (P_{crit}) and temperature to predict habitat shifts for a given species. Given that the calculated value of P_{crit} strongly depends on the rate of oxygen uptake (Rogers et al. 2016, Wood 2018), factors that substantially impact metabolic demand must be considered in designing experiments to provide ecologically relevant input data for the metabolic index model or similar approaches. The effect of grouping on metabolic rate provides an important example, particularly in regard to highly active organisms like *D. opalescens*.

Further study of the nature and extent of a potential relationship between conspecifics and energy consumption in social squids is clearly warranted. In particular, a method based on the comparison of individuals with and without conspecific simulation (Nadler et al. 2016) could be employed in conjunction with longer acclimation periods to compare RMR and basal metabolic rate measured over longer intervals.

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Appendix

Table A1. ANCOVA results. Significant factors affecting routine metabolic rate (RMR) ($p < 0.05$) in **bold**

Analysis of covariance for RMR in *Doryteuthis opalescens* with social condition as the independent variable and temperature as a covariate

Source of variation	df	MS	*F*	p
Social condition	1	0.07358	7.1448	**0.0133**
Temperature	1	0.014249	1.3836	0.251
Social condition × Temperature	1	0.00927	0.9002	0.3522
Residuals	24	0.010298		

Analysis of covariance for RMR in *Doryteuthis opalescens* with wet mass as the independent variable and the proportion of females as a covariate

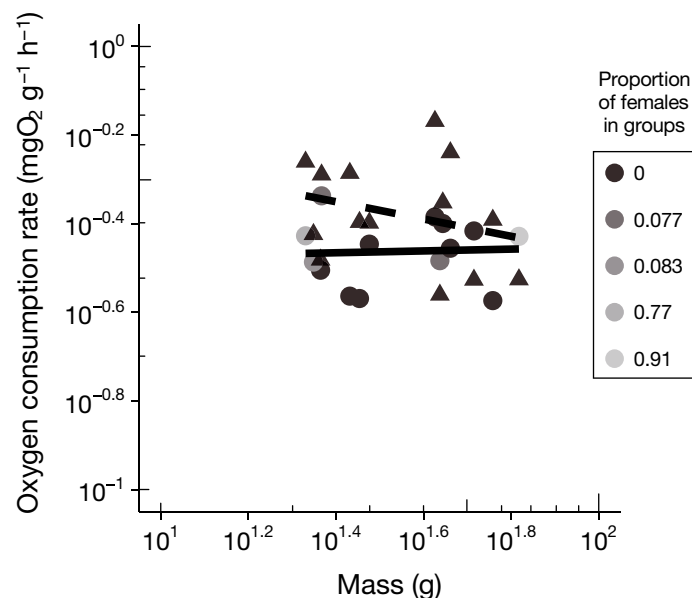| Source of variation	df	MS	*F*	p
Wet mass	1	0.000057	0.0141	0.9079
Proportion of females	1	0.001722	0.427	0.5282
Wet mass × Proportion of females	1	0.000161	0.04	0.8455
Residuals	10	0.040334		

Fig. A1. Temperature-corrected routine metabolic rate (RMR) (14°C) plotted against mass for solitary (filled triangles, dashed regression line) and grouped *Doryteuthis opalescens* (filled circles, solid regression line), with groups shaded based on the proportion of female squid (all solitary squid were male). There was a minimal negative association between solitary squid RMR and mass (slope = −0.003, p = 0.37, linear regression) and no apparent influence of sex on the neutral relationship between group RMR and mass (slope = 0.0001, p = 0.9, ANCOVA) (Table A1). In the latter comparison, mass–metabolic rate equations for each group were used to calculate rates at randomly selected masses of squid from the solitary treatment